BARD Final Report IS-4842-15 R

Proteomic investigation of a tomato receptor like protein recognizing fungal pathogens

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Project award year: 2015
Three year research project

Maximizing food production with minimal negative effects on the environment remains a long-term challenge for sustainable food production. Microbial pathogens cause devastating diseases, minimizing crop losses by controlling plant diseases can contribute significantly to this goal. All plants possess an innate immune system that is activated after recognition of microbial-derived molecules. The fungal protein Eix induces defense responses in tomato and tobacco. Plants recognize Eix through a leucine-rich-repeat receptor- like-protein (LRR-RLP) termed LeEix. Despite the knowledge obtained from studies on tomato, relatively little is known about signaling initiated by RLP-type immune receptors.

The focus of this grant proposal is to generate a foundational understanding of how the tomato xylanase receptor LeEix2 signals to confer defense responses. LeEix2 recognition results in pattern triggered immunity (PTI). The grant has two main aims: (1) Isolate the LeEix2 protein complex in an active and resting state; (2) Examine the biological function of the identified proteins in relation to LeEix2 signaling upon perception of the xylanase elicitor Eix.

1. We used two separate approaches to isolate receptor interacting proteins. Transgenic tomato plants expressing LeEix2 fused to the GFP tag were used to identify complex components at a resting and activated state. LeEix2 complexes were purified by mass spectrometry and associated proteins identified by mass spectrometry. We identified novel proteins that interact with LeEix receptor by proteomics analysis. We identified two dynamin related proteins (DRPs), a coiled coil – nucleotide binding site leucine rich repeat (SlNRC4a) protein. In the second approach we used the split ubiquitin yeast two hybrid (Y2H) screen system to identified receptor-like protein kinase At5g24010-like (SlRLK-like) (Solyc01g094920.2.1) as an interactor of LeEIX2.
2. We examined the role of SlNRC4a in plant immunity. Co-immunoprecipitation demonstrates that SlNRC4a is able to associate with different PRRs. Physiological assays with specific elicitors revealed that SlNRC4a generally alters PRR-mediated responses. SlNRC4a overexpression enhances defense responses while silencing SlNRC4 reduces plant immunity. We propose that SlNRC4a acts as a non-canonical positive regulator of immunity mediated by diverse PRRs. Thus, SlNRC4a could link both intracellular and extracellular immune perception.
3. SlDRP2A localizes at the plasma membrane. Overexpression of SlDRP2A increases the sub-population of LeEIX2 inVHAa1 endosomes, and enhances LeEIX2- and FLS2-mediated defense. The effect of SlDRP2A on induction of plant immunity highlights the importance of endomembrane components and endocytosis in signal propagation during plant immune .
4. The interaction of LeEIX2 with SlRLK-like was verified using co-immunoprecipitation and a bimolecular fluorescence complementation assay. The defence responses induced by EIX were markedly reduced when SlRLK-like was over-expressed, and mutation of *slrlk-like* using CRISPR/Cas9 increased EIX-induced ethylene production and *SlACS* gene expression in tomato. Co-expression of SlRLK-like with different RLPs and RLKs led to their degradation, apparently through an endoplasmic reticulum-associated degradation process.

We provided new knowledge and expertise relevant to expression of specific recognition specificities in plants that may be exploited to enhance immunity in crops enabling the development of novel environmentally friendly disease control strategies.

Summary Sheet

Publication Summary

PubType	IS only	Joint	US only
Reviewed	4	2	2
Thesis - MSc.	2	0	0

Training Summary

Trainee Type	Last Name	First Name	Institution	Country
Postdoctoral Fellow	Pizarro	Lorena	Tel Aviv University	Israel
Ph.D. Student	Leibman	Meirav	Tel Aviv University	Israel
M.Sc. Student	Dekel	Cohen	Tel Aviv University	Israel
Ph.D. Student	Teig	Orian	Tel Aviv University	Israel
Postdoctoral Fellow	Yadeta	Koste	University of California, Davis	USA
Ph.D. Student	Franco	Jessica	University of California, Davis	USA
M.Sc. Student	Meltz	Tal	Tel Aviv University	Israel
M.Sc. Student	Gittin	Karina	Tel Aviv University	Israel

Contribution of Collaboration

Both PIs and members of the two laboratories have met twice in person for scientific discussions as well as to exchange data and materials. Professor Coaker visited Professor Avni's laboratory in the spring of 2016. Professor Avni visiting Professor Coaker's laboratory in the summer of 2016. We met also in several scientific meetings.

Avni’s lab sent the US partner plant material and seeds for co-immunoprecipitation experiments. Coaker’s lab performed immunoprecipitation of LeEIX complexes and proteomics analyses on samples from Israel. Both laboratories exchanged results and prioritized experiments. The Avni lab is focusing on functional analyses of identified proteins with respect to tomato defense responses.

The focus of this grant proposal was to generate a foundational understanding of how the tomato xylanase receptor LeEix2 signals to confer defense responses. LeEix2 recognition results in pattern triggered immunity (PTI). The grant has two main aims: (1) Isolate the LeEix2 protein complex in an active and resting state; (2) Examine the biological function of the identified proteins in relation to LeEix2 signaling upon perception of the xylanase elicitor Eix.

1. We used two separate approaches to isolate receptor interacting proteins. Transgenic tomato plants expressing LeEix2 fused to the GFP tag were used to identify complex components at a resting and activated state. LeEix2 complexes were purified by mass spectrometry and associated proteins identified by mass spectrometry. We were able to identify several known components of receptor complexes (including the SOBIR1 receptor-like protein and the BAK1 receptor-like kinase). We also identified novel proteins that interact with LeEix receptor by proteomics analysis. We identified two dynamin related proteins (DRPs), a coiled coil – nucleotide binding site leucine rich repeat (SlNRC4a) protein. In the second approach we used the split ubiquitin yeast two hybrid (Y2H) screen system to identified receptor-like protein kinase At5g24010-like (SlRLK-like) (Solyc01g094920.2.1) as an interactor of LeEIX2.
2. We examined the role of SlNRC4a in plant immunity. Co-immunoprecipitation demonstrates that SlNRC4a is able to associate with different PRRs. Physiological assays with specific elicitors revealed that SlNRC4a generally alters PRR-mediated responses. SlNRC4a overexpression enhances defense responses while silencing SlNRC4 reduces plant immunity. Moreover, the coiled-coil domain of SlNRC4a is able to associate with LeEIX2 and is sufficient to enhance responses upon EIX perception. Based on these findings, we propose that SlNRC4a acts as a non-canonical positive regulator of immunity mediated by diverse PRRs. Thus, SlNRC4a could link both intracellular and extracellular immune perception (published in Plant Cell and Enviroment and Plant Signaling & Behavior).
3. We studied the role of dynamin related proteins (SlPRA1A), a predicted regulator of RAB, as an interactor of LeEIX2. Overexpression of SlPRA1A strongly decreases LeEIX2 endosomal localization, as well as LeEIX2 protein levels. Accordingly, the innate immune responses to EIX are markedly reduced

by SlPRA1A overexpression, presumably due to a decreased LeEIX2 availability. Studies into the role of SlPRA1A in LeEIX2 trafficking revealed that LeEIX2 localization in multivesicular bodies/late endosomes is augmented by SlPRA1A. Furthermore, inhibiting vacuolar function prevents the LeEIX2 protein level reduction mediated by SlPRA1A, suggesting that SlPRA1A may redirect LeEIX2 trafficking to the vacuole for degradation. Interestingly, SlPRA1A overexpression reduces the amount of several RLP-PRRs, but does not affect the protein level of receptor like kinase PRRs, suggesting a specific role of SlPRA1A in RLP-PRR trafficking and degradation (published in Frontiers of Plant Sciences).

4. We identified SlRLK as an interactor of LeEix2. The interaction of LeEIX2 with SlRLK-like was verified using co-immunoprecipitation and a bimolecular fluorescence complementation assay. The defence responses induced by EIX were markedly reduced when SlRLK-like was over-expressed in *Nicotiana benthamiana* or *Nicotiana tabacum*, and mutation of *slrlk-like* using CRISPR/Cas9 increased EIX-induced ethylene production and *SlACS* gene expression in tomato. Co-expression of SlRLK-like with different RLPs and RLKs led to their degradation, apparently through an endoplasmic reticulum-associated degradation process. Moreover, SlRLK-like associated with RLK FLS2 resulting in a reduction in the flg22-induced burst of reactive oxygen species. Our findings suggest that SlRLK-like negatively regulates defence responses by targeting various PRRs for degradation.

Agriculture impact

Editing SlNRC4a by Crispr/Cas, result in a 67 aa truncated protein. This line showed enhanced immune responses (published in Plant Cell and Environment), and may be of use in commercial varieties.

In aim, 1 of the proposal we suggested to identify loosely bound proteins to the LeEix2 receptor by labeling them with promiscuous biotin ligase. This approach, which was very promising, did not succeed and we concentrated on co-immunoprecipitation and yeast two-hybrid screening.

Publications for Project IS-4842-15 R

Status	Type	Authors	Title	Journal	Vol:pg Year	Coun
Accepted	Thesis - MSc.	*Dekel Cohen*	Characterization of NbMAP3k-3like, a novel candidate in the LeEIX2/EIX signaling pathway		: 2017	IS only
Published	Reviewed	*Lorena Pizarro, Meirav Leibman-Markus, Silvia Schuster, Maya Bar, Tal Meltz, Adi Avni*	Tomato Prenylated RAB Acceptor Protein 1 Modulates Trafficking and Degradation of the Pattern Recognition Receptor LeEIX2, Affecting the Innate Immune Response	***Front Plant Sci***	9 : 257 2018	IS only
Published	Reviewed	*Zhang Meixiang, Coaker Gitta*	Harnessing Effector-Triggered Immunity for Durable Disease Resistance	***Phytopathology***	107 : 912-919 2017	US only
Published	Reviewed	*Michelmore R, Coaker G, Bart R, Beattie G, Bent A, Bruce T, Cameron D, Dangl J, Dinesh-Kumar S, Edwards R, Eves-van den Akker S, Gassmann W, Greenberg JT, Hanley-Bowdoin L, Harrison RJ, Harvey J, He P, Huffaker A, Hulbert S, Innes R, Jones JDG, Kaloshian I, Kamoun S, Katagiri F, Leach J, Ma W, McDowell J, Medford J, Meyers B, Nelson R, Oliver R, Qi Y, Saunders D, Shaw M, Smart C, Subudhi P, Torrance L, Tyler B, Valent B, Walsh J*	Foundational and Translational Research Opportunities to Improve Plant Health	***Mol Plant Microbe Interact***	30 : 515-516 2017	US only
Published	Reviewed	*Leibman-Markus, M., Pizarro, L., Bar, M., Coaker, G., and Avni, A*	NRC proteins - a critical node for pattern and effector mediated signaling.	***Plant Signal Behav***	13 : e1507404 2018	Joint
Published	Reviewed	*5. Pizarro, L., Leibman-Markus, M., Schuster, S., Bar, M., and Avni, A.*	SlPRA1A/RAB attenuate EIX immune responses via degradation of LeEIX2 pattern recognition receptor	***Plant Signal Behav***	13 : e1467689 2018	IS only
Published	Reviewed	*2. Leibman-Markus, M., Pizarro, L., Schuster, S., Lin, Z.J.D., Gershony, O., Bar, M., Coaker, G., and Avni, A.*	The intracellular nucleotide-binding leucine-rich repeat receptor (SlNRC4a) enhances immune signalling elicited by extracellular perception	***Plant Cell Environ***	41 : 2313-2327 2018	Joint
Published	Reviewed	*Meirav Leibman-Markus, Silvia*	LeEIX2 Interactors' Analysis and EIX-Mediated Responses	***Methods Mol Biol***	1578 : 167-172	IS only

		Schuster, and Adi Avni	Measurement		2017	
Accepted	Reviewed	*Pizarro, L., Leibman-Markus, M., Schuster, S., Bar, M. and Avni, A.*	Tomato Dynamin Related Protein 2A associates with LeEIX2 and enhances PRR mediated defense by modulating receptor trafficking	***Front Plant Sci***	: 2019	IS only
Published	Thesis - MSc.	*Karina Gitin*	Investigating tomato receptor-like protein (RLP) signaling during plant innate immunity		: 2019	IS only